The Effect of Foster-Nursing on Mouse Lymph Nodes*

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SUMMARY

The effect of foster-nursing on mouse lymph node size and metabolism was investigated. Newborn mice of the C3H/AnSp, C3Hf/AnSp, and PL/JaxSp strains, which characteristically have large lymph nodes, were foster-nursed by mothers from strains characterized by small lymph nodes (C57BL/Sp and AKR/JaxSp), and vice versa. The mice were sacrificed at 6½–7½ weeks of age, and the two elbow and two inguinal nodes were removed, pooled, and weighed. Protein metabolism of the lymph nodes was studied in vivo by measuring the uptake of radioactivity following the administration of DL-lysine-2-C14.

The foster-nursing of mice from “large lymph node” strains by mothers possessing small nodes resulted in a diminution of lymph node size. On the other hand, the foster-nursing of mice with small lymph nodes by mothers with large lymph nodes resulted in large lymph nodes in the young. These changes were independent of body weight differences.

Foster-nursing altered the protein metabolism of the lymph nodes. This was not confined to lymph node protein, however, inasmuch as kidney and liver protein metabolism were also affected.

Recent experiments in our laboratory1 have indicated that the lymph nodes of virgin C3H mice with a high cancer incidence are larger than those of mice of other strains possessing a lower cancer incidence. These differences obviously may be genetic in origin. On the other hand, an explanation for them might be found in the work with germ-free animals (review by Luckey [9]), which suggests that lymphatic tissue develops poorly in the absence of an exogenous stimulation. If this is the case, the fact that mouse lymph nodes grow rapidly during the nursing period and the few weeks immediately following weaning1 might indicate that a growth stimulus is obtained via the milk and that it differs from one strain to another.

This possibility was examined in a series of foster-nursing experiments. Mice from strains possessing relatively large lymph nodes were foster-nursed by females from strains that characteristically possessed small lymph nodes, and vice versa. The results of these experiments are reported here.

MATERIALS AND METHODS

Foster mothers were mice of the C3H/AnSp, C3Hf/AnSp, PL/JaxSp, AKR/JaxSp, or C57BL/Sp strains, obtained from the inbred stocks maintained in the laboratories of the Detroit Institute of Cancer Research. Mice to be foster-nursed were females of these strains obtained by first-generation pen-breeding. The newborn mice remained with their mothers from 0 to 18 hours before being transferred to foster mothers. They were weaned after 5 weeks of foster-nursing and thereafter were given the stock diet and water ad libitum.

All mice were housed in either metal or wooden cages, with five to ten animals per cage, and kept on racks in an air-conditioned room. The animals were sacrificed by cervical dislocation at 6½–7½ weeks of age. A complete autopsy was performed on each animal, and those whose state of health was doubtful were discarded. The two inguinal and

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the two elbow nodes were removed, blotted, and weighed on a Roller-Smith torsion balance. In some animals the spleen was also removed and weighed.

DL-Lysine-2-C\textsuperscript{14} was administered to some of the mice 6 hours prior to sacrifice for the purpose of studying its uptake by lymph node proteins. The protein was obtained from the lymph nodes and prepared for radioactivity assay in the following manner. The nodes were extracted with trichloroacetic acid and subsequently with hot ethanol-ether as described previously. The protein thus obtained was re-homogenized in distilled water. A measured aliquot was spread uniformly on a disc of Whatman No. 542 filter by means of a stainless steel filter tower apparatus, and washed repeatedly with acetone. The samples were then mounted on an aluminum ring and disc assembly and assayed for radioactivity in a windowless gas flow counter. The filter papers were placed in tubes, a measured amount of 1 N NaOH was added, and the tubes were placed in a water bath maintained at 80° C. for 20 minutes. An aliquot of this solution was used for the determination of protein.

\textbf{RESULTS}

Lymph node and body weights of the mice are shown in Table 1. Strain differences in lymph nodes were readily apparent in the animals that were nursed by their natural mothers. The C3H/AnSp, PL/JaxSp, and C3Hf/AnSp mice possessed large nodes, and the C57BL/Sp and AKR/JaxSp had smaller ones. There was very little difference between the body weights of the C3H/AnSp, C57BL/Sp, PL/JaxSp, and AKR/JaxSp strain mice. C3Hf/AnSp strain mice weighed the most.

The control animals were mice that had been foster-nursed by mothers of their own strain. Foster nursing in this way resulted in smaller lymph nodes in the C3Hf/AnSp strain mice and was without effect on the lymph node size of any of the other strains.

When mice of the C57BL/Sp strain were foster-nursed by females of the AKR/JaxSp, PL/JaxSp, or C3Hf/AnSp strains, their lymph nodes were in-

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
\textbf{Strain} & \textbf{Foster mother} & \textbf{No.} & \textbf{Body weight} & \textbf{Lymph node weight} \\
 & & \textbf{animals} & \textbf{(g.m.)} & \textbf{(mg.)} \\
\hline
C3H/AnSp & C3H/AnSp & 19 & 14.5 ± 0.4 & 19.7 ± 1.1 \\
C3Hf/AnSp & C3H/AnSp & 17 & 17.3 ± 0.4 & 19.7 ± 0.9 \\
C57BL/Sp & C57BL/Sp & 18 & 15.1 ± 0.4 & 10.5 ± 0.5 \\
PL/JaxSp & PL/JaxSp & 19 & 15.6 ± 0.3 & 91.9 ± 0.9 \\
AKR/JaxSp & PL/JaxSp & 19 & 14.6 ± 0.6 & 12.7 ± 0.9 \\
PL/JaxSp & PL/JaxSp & 19 & 16.7 ± 0.4 & 22.5 ± 1.1 \\
1. PL/JaxSp & C57BL/Sp & 20 & 17.0 ± 0.5 & 16.5 ± 1.0 \\
2. PL/JaxSp & C57BL/Sp & 15 & 15.4 ± 0.6 & 13.7 ± 0.9 \\
PL/JaxSp & AKR/JaxSp & 15 & 13.9 ± 0.3 & 15.0 ± 0.8 \\
C3Hf/AnSp & C3Hf/AnSp & 20 & 14.7 ± 0.7 & 13.0 ± 1.0 \\
C3H/AnSp & C3H/AnSp & 20 & 15.5 ± 0.6 & 16.0 ± 1.1 \\
1. C3Hf/AnSp & C57BL/Sp & 20 & 14.6 ± 0.5 & 12.5 ± 0.7 \\
2. C3Hf/AnSp & C57BL/Sp & 11 & 14.7 ± 0.4 & 11.6 ± 0.5 \\
C57BL/Sp & C57BL/Sp & 19 & 13.3 ± 0.5 & 10.1 ± 1.1 \\
C57BL/Sp & AKR/JaxSp & 20 & 13.7 ± 0.6 & 14.3 ± 1.1 \\
1. C57BL/Sp & C3Hf/AnSp & 17 & 13.7 ± 0.5 & 13.8 ± 1.0 \\
2. C57BL/Sp & C3Hf/AnSp & 10 & 14.2 ± 0.5 & 16.4 ± 1.8 \\
C57BL/Sp & PL/JaxSp & 17 & 14.2 ± 0.5 & 18.2 ± 1.5 \\
\hline
\end{tabular}
\caption{Lymph Node Weights in Foster-Nursed Mice*}
* Figures represent mean values and standard errors of the means.
creases in the uptake of C\textsuperscript{14} by the protein of lymph nodes and kidneys, and no change in that in the liver protein (Table 2). It produced an increased protein content in the kidneys and liver, but did not change the protein concentration in the lymph nodes.

The changes that were observed in spleen weights, with foster-nursing, seemed to be correlated with changes in the body weight. In two cases spleen weights were decreased. In the first, the spleens of C57BL/Sp mice foster-nursed by mothers of the same strain were reduced from 78.1 ± 8.9 to 55.7 ± 5.0 mg. In the second, spleen weights of PL/JaxSp mice foster-nursed by AKR/JaxSp mothers were reduced from 53.6 ± 4.3 to 35.5 ± 1.2 mg. In both cases the foster-nursed animals weighed less than the controls (Table 1).

**DISCUSSION**

Foster-nursing is a procedure that has been widely used by investigators to determine the role of the milk as a source of extrachromosomal factors in spontaneous tumorigenesis (1, 4, 8, 10) and in tumor transplantation (8). Maternal influences exerted during the nursing period have been found to affect longevity (2, 10), body weight (3, 5), mammary gland morphology (7), and endocrine organs (11).

It can be inferred from the work with germ-free animals (9) that in the absence of an outside stimulus lymphatic tissue fails to develop to an appreciable extent. Presumably the amount of development that takes place depends upon the amount of stimulation that has occurred. Undoubtedly exogenous microorganisms provide a portion of the stimulus responsible for the growth of lymph nodes, and the results of the present experiments suggest that contact with the mother can provide an additional stimulus. The extent of this stimulation may be relatively great, as in the case of the PL/JaxSp females, or small, as in the case of the C57BL/Sp mice.

In considering the milk as a factor stimulating lymph node development, one considers first the milk proteins that might be antigenic for the young of a foreign strain. The decrease in lymph node weight seen in PL/JaxSp mice that were foster-nursed by C57BL/Sp and AKR/JaxSp strain mice, and in C3Hf/AnSp mice that were foster-nursed by mothers of their own strain, suggests that milk probably exerts no noticeable antigenic effect as measured by the criterion of lymph node size. The possibility must then be considered that the milk from one strain carries a substance (or substances) that is peculiar to the strain from which the foster mother is derived. An example of this is the well known mammary tumor incitor agent, and there may be other factors present that have not yet been identified by known effects.

Another explanation might be that the changes in lymph node weights are due to a different nutritional state of the animals brought about by the removal of the natural mother, inasmuch as foster-nursing has been shown to affect body weight (3, 5). If such is the case, there should be a direct relationship between changes in lymph node size and

**TABLE 2**

<table>
<thead>
<tr>
<th></th>
<th>PL/JaxSp controls</th>
<th>PL/JaxSp foster-nursed by C57BL/Sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. animals</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (gm.)</td>
<td>15.4 ± 0.7</td>
<td>15.8 ± 0.4</td>
</tr>
<tr>
<td>Lymph nodes:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (mg.)</td>
<td>23.0 ± 0.6</td>
<td>15.1 ± 0.7</td>
</tr>
<tr>
<td>Protein (µg/mg tissue)</td>
<td>70 ± 2</td>
<td>65 ± 4</td>
</tr>
<tr>
<td>C\textsuperscript{14} (counts/min/mg protein)</td>
<td>0.31 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>Kidney:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (mg.)</td>
<td>191 ± 8</td>
<td>123 ± 7</td>
</tr>
<tr>
<td>Protein (µg/mg tissue)</td>
<td>97 ± 10</td>
<td>125 ± 2</td>
</tr>
<tr>
<td>C\textsuperscript{14} (counts/min/mg protein)</td>
<td>0.41 ± 0.02</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Liver:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein (µg/mg tissue)</td>
<td>137 ± 9</td>
<td>191 ± 4</td>
</tr>
<tr>
<td>C\textsuperscript{14} (counts/min/mg protein)</td>
<td>0.19 ± 0.03</td>
<td>0.16 ± 0.01</td>
</tr>
</tbody>
</table>

* See footnote Table 1.
changes in body weight. Decreases in lymph node weights were sometimes, but not always, accompanied by smaller body weights, and increases in lymph node weights were never accompanied by larger body weights. Thus, alterations in the nutritional state that are brought about by the presence of a foster mother do not appear to be a major factor in the lymph node changes described in these experiments.

The experiments described here do not rule out the possibility that an underlying genetic control may determine the reactivity of the lymph nodes during foster-nursing. This is presently under investigation.

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